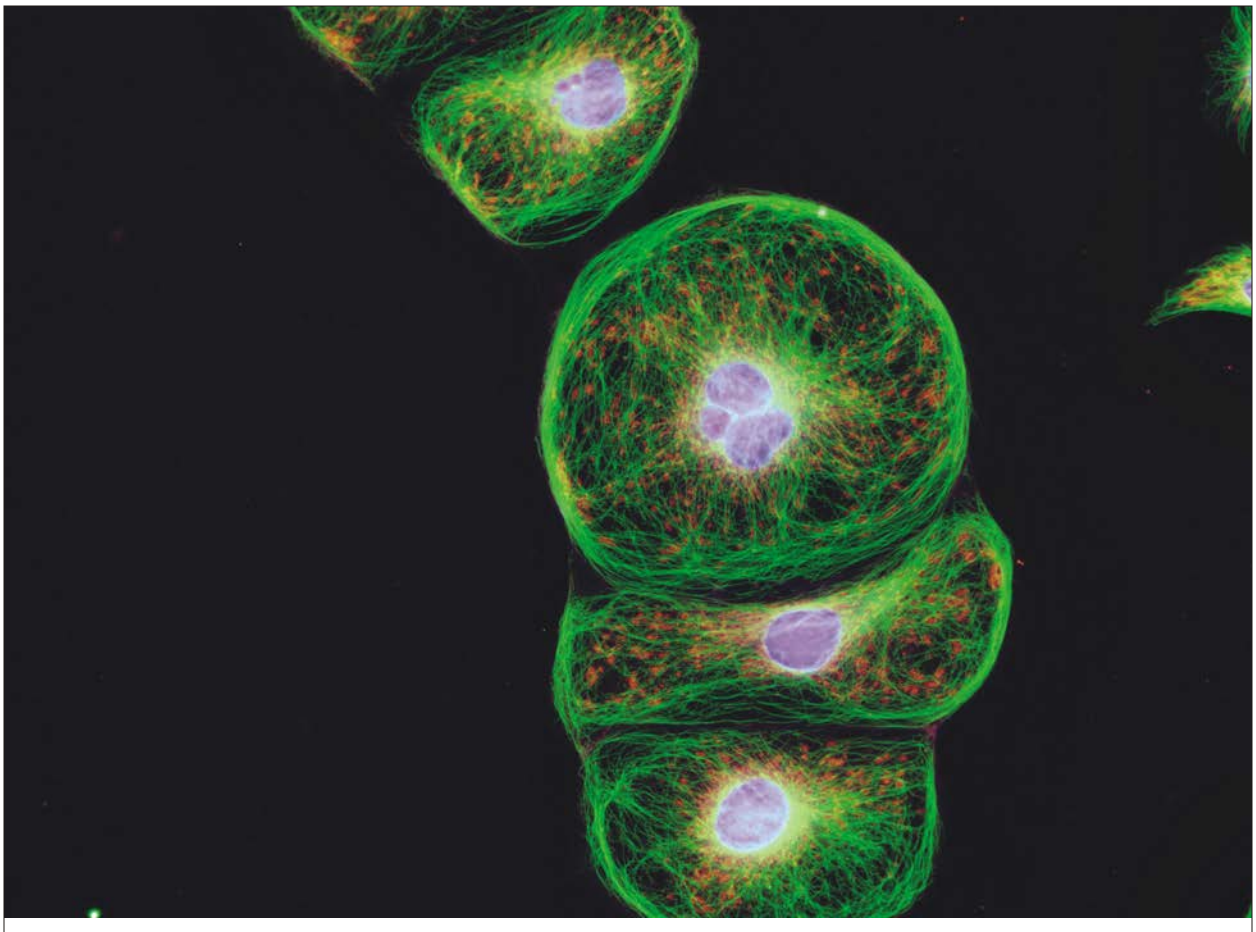

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Evaluation of Polymerase Chain Reaction for Rapid Detection of *E. coli* Strains: A Preliminary Study

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Abstract: *E. coli* strains were detected in environmental samples using conventional methods and the Polymerase Chain Reaction (PCR). The organism was isolated by conventional bacteriological isolation and identification. The identity of the nucleic acid sequence of the organism was confirmed using PCR based detection assay. In the PCR assay, a pair of primers derived from uidA gene of *E. coli*, encoding glucuronidase specific for *E. coli* was used. Conventional isolation and identification is the most accurate method for detection of an active (intact) organism in environmental samples. However, this method is tedious, laborious and time consuming. The PCR provides a reliable, rapid, sensitive and specific assay for monitoring *E.coli* strains. The results of this study indicated that PCR should be used as a routine diagnostic technique for rapid detection of *E.coli* in environmental samples.

Key words: *E. coli*, environment, diagnositcs, PCR

Introduction

Strains of *E. coli* were recognized as a cause of gastroenteritis. They are important causes of childhood diarrhea in developing countries (Moss and Adams, 2000). Pollution from human and animal waste is traditionally indicated by the presence of commensal *E. coli*. Though, these organisms are essentially non-pathogenic, their presence warns of the possible concurrent existence of pathogenic microbes (Carson *et al*., 2003). Complete detection and identification of *E. coli* by conventional methods is too complicated for routine use. To address these problems, different tests have been evolved for identifying the organism rapidly with a high degree of certainty. Some of these methods have been standardized at international and national levels and accepted for routine use, whereas others are still in the developmental or evaluative stage.

Recently, efficient cultivation media for routine monitoring of *E. coli* in environmental freshwaters have been developed. Those methods are generally applied in a quantitative way to estimate the actual concentration of *E. coli*. Differentiation is not the main purpose for most routine applications, although there is a considerable demand for techniques for the simultaneous detection and differentiation of *E. coli* populations in aquatic habitats. A practical method generating a representative genetic fingerprint of population structures would enable routinely performed qualitative investigations such as the comparison of different sources of faecal emissions (Farnleitner *et al*., 1999). More recently, the PCR has been proliferated because of its simplicity,

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rapidity, reliability, reproducibility, sensitivity and specificity for monitoring of microorganisms. PCR is a molecular- biological process that, during recent years, has been developed into a method used in virtually every area of medicine and natural sciences. PCR proved satisfactory to support the conventional bacteriological methods and DNA hybridization technique (Aradaib and Ali, 2004). In the present investigation, PCR assay was evaluated for detection and differentiation of *E. coli* strains in environmental samples.

Materials and Methods

Collection of Environmental Samples

Suspected environmental samples of containing *E. coli* strains were collected in clean sterile bottles from different environmental sources in Khartoum State. The samples were collected from different sources of water including animal houses, drinking water, stagnant water and sewage as well as from animal and human faeces.

Conventional Isolation and Identification

Initially suspected samples from human and animal faeces were grown on MacConkey's medium as selective and differential medium to exclude non-lactose fermenter of the family Enterobacteriaceae. Lactose is included as a fermentable carbohydrate with a pH indicator, usually neutral red. Strong acid producers like *Escherichia*, *Klebsiella* and *Enterobacter* produced red colonies. Loopful of suspension from each medium was streaked on Eosin Methylene Blue (EMB) agar, plates were incubated 18-24 h at 37°C examined for typical *E. coli* colonies, dark centered with or without metallic sheen. Two typical colonies from each EMB plate were picked and transferred to nutrient agar slants for morphological and biochemical tests. Slants were incubated at 37°C for 18-24 h. Gram stain on each culture was performed, for all cultures appearing as Gram- negative, short rods or cocci, were identified by Triple Sugar Iron (TSI) biochemical scheme, motility test, indole test, urase test, lysine decomposition test and DNAse hydrolysis test (Colle *et al*., 1996). The rest of the samples were isolated using multiple tube technique and identified as described previously (Geldreich, 1975).

Extraction of Bacterial DNA

Purified colonies of isolated strains of *E. coli* were each transferred to epindorf tubes containing 300 µL of double distilled water, using a bacteriological loop. The colonies were resuspended in distilled water by vortexing. DNAs were extracted from bacterial samples by boiling the tubes in a water bath at 100°C for 30 min. The epindorf tubes were then centrifuged at 12,000 rpm for 15 min and the supernatant containing DNAs was transferred to new tubes and stored at -20°C until used for PCR amplification.

Primers Selection

A pair of oligonucleotide primers (P1 and P2) was derived from a highly conserved region of nucleotide sequences of the uidA gene of *E. coli*, encoding-glucuronidase specific for *E. coli* (Juck *et al*., 1996). Primers (20 mer each) Primers 1 and 2 (P1 and P2) were selected for the synthesis of specific PCR product. P1 (5'-ATC ACC GTG GTG ACG CAT GTC GC-3') included 23 bases of the positive sense strand. P2 (5'-CAC CAC GAT GCC ATG TTC ATC TGC-3') included 24 bases of the complementary strand. The PCR using primer P1 and P2 would result in a 486 bp PCR product. All primers were synthesized on a DNA synthesizer (Milligen/Biosearch, a division of

MilliporeBurlington, MA, USA) and purified using oligo-pak oligonucleotide purification columns (Glen Research Corporation, Sterling, Virginia, USA).

Polymerase Chain Reaction (PCR)

A stock buffered solution containing (250 μL) 10X PCR buffer, 100 μL of 125 mM Mg Cl_2, 12.5 μL of each dNTPs (ATP, TTP, GTP and CTP) at a concentration of 10 mM) was prepared in 1.5 mL tube. The primers were used at a concentration of 20 micromole per liter (L). Double distilled water was added to bring the volume of the stock buffer solution to 1.5 mL. For each PCR amplification, 2.0 μL of the target DNA and 2 μL of primers was added to 45 μL of the stock solution in PCR tubes and mixed by vortexing. 1.0 μL of Taq DNA polymerase (Perkin Elmer) was used at a concentration of 5.0 units. All PCR amplification reactions were carried out in a final volume of 50 μL. The thermal cycling profiles were as follows: a 2 min incubation at 94°C, followed by 30 cycles of 94°C for 1 min 57°C for 30 sec and 72°C for 45 sec. A final incubation at 72°C for 10 min was carried out to ensure complete synthesis of the expected PCR products.

Thermal profiles were performed on a Techne PHC-2 thermal cycler (Techne, Princeton, NJ.). Following amplification, 20 μL from each PCR reaction containing amplified product were loaded onto gels of 1.0% SeaKem agarose (FMC Bioproduct, Rockland ME) and electrophoresed. The gels were stained with ethidium bromide and the PCR products were identified following visualization under UV light.

Results and Discussion

Gram-negative bacteria morphology resembling those of *E. coli* was encountered in impression smears of purified colonies. Confluent bacterial growth of pink colonies was evident in MacConkey's media. Characterization and identification of isolated *E. coli* strains was made possible by biochemical reactions. In the PCR assay, the pair of primers (P1 and P2) afforded sensitive and specific detection of a 486 base pair (bp) PCR product from *E. coli*. The sensitivity of the PCR assay indicated that the

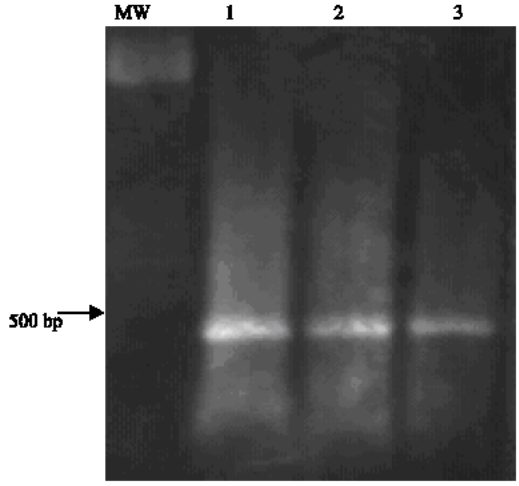

Fig. 1: Sensitivity of the Polymerase Chain Reaction (PCR) for detection of the specific 486 bp PCR product from Sudanese isolate of *E. coli*. Lane MW: Molecular Weight marker; Lanes 1-3: (*E. coli* DNA extract) 100, 10 and 1.0 pg, respectively

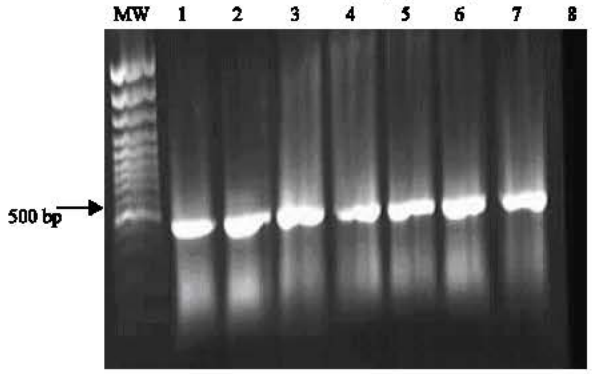

Fig. 2: The specific 486 bp-PCR product from 1.0 pg DNA of different isolates of *E. col* Lane MW: Molecular Weight marker; Lane 1-7: DNA extracted from different *E. coli* isolates. Lane 8: DNA (negative control)

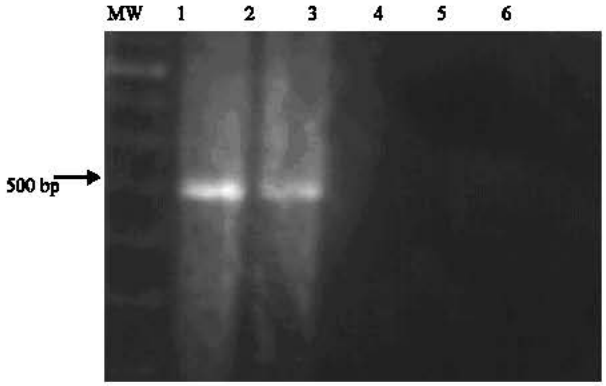

Fig. 3: Specificity of the PCR for detection of *E. coli* strains palyam serogroup of orbiviruses RNA. Amplification product was not detected from nucliaieic acid-free samples. Lane MW: Molecular Weight marker; Lane 1and 2: 1.0 pg of DNA extract from *E. coli* (positive controls); Lane 3-6: DNA-free sample

specific 486 bp PCR product was visualized on ethidium bromide-stained gel from 1 picogram DNA of isolated bacteria (Fig. 1). The specific PCR products were also detected in a number of *E. coli* strains isolated from a variety of environmental samples (Fig. 2). The specificity studies indicated that, the specific PCR product was not amplified from nucleic acid-free control samples (Fig. 3).

E. coli is commonly found in the environment, including all natural waters and soils that are subject to recent faecal contamination, whether from humans, agriculture, or wild animals and birds. It is not difficult, however, to demonstrate the presence of *E. coli* in air, in dust, on the hand and in many foods (Jay, 1986). Recently, it has been suggested that *E. coli* may be found or even multiply in tropical waters that are not subjected to human faecal pollution. However, even in the remotest regions, faecal contamination by wild animals, including birds, can never be excluded. As animals can transmit pathogens infective for humans, the presence of *E. coli* or thermotolerant coliform bacteria can never be excluded (Anonymous, 1995).

To advance beyond the current knowledge of the biology and ecology of the bacteria, we have evaluated PCR assay for detection of *E. coli* in environmental samples from different areas of Khartoum State, Sudan.

The result of this study indicated that the PCR could be used as an alternative or supportive diagnostic assay to the lengthy cumbersome conventional bacterial isolation and identification procedure. The rapidity, sensitivity and specificity of the PCR assay would greatly facilitate rapid detection of *E. coli* in environmental samples (Heininger *et al*., 1999). Because the PCR amplification assay is an extremely sensitive procedure, care must be taken to avoid cross-contamination between tubes during pipetting of reagents. Negative and positive controls should be included in each PCR reaction to estimate the lower limit of specificity and the higher limit of sensitivity. It is recognize that samples from Khartoum State are representative and not from the whole country, the Sudan, but it is enough for a report describing the application of PCR for detection of *E. coli* in environmental samples in comparison to conventional bacteriological methods.

Further studies are currently under way to increase the sensitivity of this PCR assay by using internal primers in a nested PCR (nPCR) format. The developed nPCR will be evaluated for direct detection of *E. coli* in environmental samples. In addition, the diagnostic potential of the nPCR will also be compared with current methods used for quantification of *E. coli* strains.

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